Supplementary Online Content

Smith JG, Luk, K, Schulz CA, et al. Association of low density lipoprotein cholesterol related genetic variants with aortic valve calcium and incident aortic stenosis. *JAMA*. doi:10.1001/jama.2014.13959.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

A. Risk factor definitions in the MDCS

In the Malmö Diet and Cancer study (MDCS), smoking was defined as self-reported regular smoking or smoking cessation within the last year. Diabetes mellitus was defined as a self-reported physician's diagnosis or use of anti-diabetic medications, or from one of five nation-wide or regional registers. Hypertension was defined as use of antihypertensive medication or blood pressure ≥140/90 mmHg.

B. Genotyping in the MDCS

In the MDCS, DNA extracted from peripheral blood cells was assigned to batches without regard to aortic stenosis status or personal identity. Batches were genotyped for all 116 single nucleotide polymorphisms (SNPs) associated with plasma concentrations of LDL-C, HDL-C or TG in the Global Lipid Genetics consortium study[1] with the same set of reagents using a MALDI-TOF mass spectrometer (Sequenom MassArray, Sequenom, San Diego, CA) using Sequenom reagents and protocols with 10 ng DNA as PCR template. Automatic allele calls by the Sequenom software were validated by manual inspection. For SNPs where commercial primers were unavailable, proxy SNPs were identified using SNAP version 2.2.[2] In only four cases were no proxy found (rs261342 and rs10761731 associated with TG, rs1084651 associated with HDL-C, rs217386 associated with LDL-C). All proxy SNPs had a r^2 higher than 0.8, except for two SNPs (rs3764261, r^2=0.48; rs9488822, r^2=0.68). The SNPs included in each score are summarized in Supplementary tables 4-6. Detailed information about proxy SNPs used is shown in Supplementary table 7. A total of 21 SNPs which failed genotyping were genotyped individually using real-time polymerase chain reaction (PCR) with 2.5 ng DNA as PCR template for allelic discrimination on an ABI 7900HT (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Genotype calls for SNPs genotyped using ABI 7900HT were obtained using SDS 2.3 software (Life Technologies, Carlsbad, CA, USA) and fluorescence intensity plots were manually inspected and curated. Prespecified DNA quality metrics included ≥60% successfully genotyped SNPs. Prespecified SNP quality metrics included success rate >90% (SNPs rs4148008, rs4731702, rs11136341, rs10195252) and Hardy-Weinberg Equilibrium p-value $>5.7x10^{-4}$ (SNPs rs386000, rs6065906, rs838880, rs7206971, rs3850634, rs2807834, rs13238203). The rs10455872 was excluded from all GRS due its known association with Lp(a).The final scores included 31 SNPs for LDL, 41 SNPs for HDL and 26 SNPs for TG.

C. Ascertainment of aortic stenosis in the MDCS

Prevalent and incident diagnoses of aortic stenosis or aortic valve replacement were identified by record linkage to national registers using personal identification numbers,[3] The Swedish Hospital Discharge Register (HDR) and the Swedish Cause of Death Register (CDR).[4] Both registers are administered by the Swedish National Board of Health and Welfare. Data collection in the HDR was started in the 1960s and includes dates of admission and discharge as well as primary and contributory diagnoses from all public hospitals in Sweden. Reporting to the HDR has been compulsory since 1987 but the only hospital in Malmö (Malmö University Hospital) has reported since 1969. The CDR includes diagnoses from death certificates since 1952, regardless if death occurred outside of Sweden. Diagnoses in the HDR are coded as primary or contributory and in the CDR as underlying or contributory cause of death, both using the International Classification of Disease (ICD). The 8th edition (ICD-8) was used until the end of 1986, the 9th edition (ICD-9) between 1987 and 1996 and the 10th edition (ICD-10) from 1997 until present. Surgical procedures in the HDR are coded based on a Swedish adaptation of the classification of surgical procedures from the Nordic Medico-Statistical Committee (NOMESCO), and used the Op6 classification from 1963 until 1989 and the KKÅ classification since then.

Aortic stenosis was defined as diagnosis codes 424.10, 424.11, 424.19 (ICD-8), 424B, 424BA, 424BB (ICD-9), I35.0 or I35.2 (ICD-10) as primary or contributory diagnosis. Aortic valve replacement for aortic stenosis was

defined as a diagnosis code of AS in combination with a diagnosis code for aortic valve replacement; FMA or FMD for KKÅ and 3074, 3075, 3116, 3117 or 3078 for Op6.

High diagnostic validity of aortic stenosis in national Swedish registers has previously been confirmed, and most patients with an AS diagnosis in national registers had moderate to severe AS.[5]

D. Genetic lipid score analyses in CHARGE

In the cohorts from the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium,[6-8] we employed a method that utilizes summary-level GWAS data for individual SNPs to efficiently calculate a β-coefficient and SE (β_{GRS} and SE_{GRS}) for the association between the GRS and presence of AVC. This method calculates the β_{GRS} as a weighted average of the β coefficients for the association between each lipid SNP and AVC in the GRS weighting each SNP by the β coefficient for the association between that SNP and the lipid trait. Weights (i.e β coefficients and standard errors for the association between each of the lipid SNPs and their lipids traits) were obtained from published GWAS reports of LDL-C, HDL-C and TG.[9]
In this analysis, we want to calculate $\hat{\alpha}$, an estimator of α in the following equation, using *m* SNPs:

$$\eta_i = r_i \alpha$$

Where r_i is the lipid trait genetic risk score for the i^{th} subject and η_i is the AVC status for the i^{th} subject. To do so, we calculate $\hat{\alpha}$ and its standard error using the equations as described by Ehret et al[10] given by:

$$\tilde{\alpha} = \frac{\sum_1^m w_j \ \hat{\beta}_j S_j^{-2}}{\sum_1^m w_j^2 S_j^{-2}}$$

$$\mathrm{SE}(\tilde{\alpha}) = \sqrt{\frac{1}{\sum_1^m w_j^2 S_j^{-2}}}$$

where β_j is the published effect size estimate when AVC is regressed onto the j^{th} SNP for i^{th} subject (x_{ij}) and its standard error (S_j) and, w_j a weight determined by the published effect size estimate when the lipid trait is regressed onto SNP x_{ij}.

Thus when all SNPs used in the risk score are uncorrelated we have that $\hat{\alpha} \cong \tilde{\alpha}$ under the null hypotheses that the $\hat{\beta}_j$ are normally distributed with mean zero and variance equal to S_j^2 and the $\tilde{\alpha}$ are normally distributed with mean zero and variance equal to $\mathrm{SE}(\tilde{\alpha})$.

These analyses were performed using the Genetics ToolboX (gtx) R package version 0.08 (http://cran.r-project.org/web/packages/gtx/index.html), we report odds ratio with confidence interval and p-value for the associations between AVC and the genetic risk scores for LDL-C, HDL-C and TG . We report odds ratio with confidence interval and p-value for the associations between AVC and per unit increment in the GRS for LDL-C, HDL-C and TG.

E. Multivariable Mendelian Randomization

In this analysis, for each SNP in a given genetic score, the β coefficient for the association with AVC (β_{AVC}) was regressed on the β coefficient for the association with the main lipid trait (β_{lipid}) using a linear regression model. For these analyses, β_{LDL}, β_{HDL} and β_{TG} estimates were obtained from a recent report by Willer et al.[9] The regression model was then adjusted for the β coefficients for associations with a secondary lipid trait for that SNP. For example, when we evaluated the regression of β_{LDL} with β_{AVC}, we adjusted for the β coefficients for secondary associations with HDL-C (β_{HDL}) and TG (β_{TG}) of all included SNPs.

eReferences

1. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010; 466(7307): 707–13.
2. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; 24(24): 2938–9.
3. Ludvigsson JF, Otterblad-Olausson P, Pettersson BU, Ekbom A. The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research. *Eur J Epidemiol* 2009; 24(11): 659–67.
4. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011; 11(1): 450.
5. Thanassoulis G, Campbell CY, Owens DS, et al. Genetic associations with valvular calcification and aortic stenosis. *N Engl J Med* 2013; 368(6): 503–12.
6. Bild DE, Bluemke DA, Burke GL, et al. Multi-ethnic study of atherosclerosis: objectives and design. Am J Epidemiol. 2002;156(9):871-881.
7. Dawber T, Meadors GF, Moore FE. Epidemiologic approaches to heart disease: the Framingham study. American Journal of Public Health. 1951;41:279-286.
8. Harris TB, Launer LJ, Eiriksdottir G, et al. Age, Gene/Environment Susceptibility-Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol 2007;165:1076-87.
9. Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013; 45(11): 1274–83.
10. Ehret GB, Munroe PB, Rice KM, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 2011; 478(7367): 103–9.
11. Teslovich TM, Musunuru K, Smith AV, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. Aug 5 2010;466(7307):707-713.

eTable 1. Components of the Genetic Score for LDL-C in CHARGE

Locus (closest gene)	SNP	n	Alleles Major/minor (MAF)	Weight	Coded Allele	Effect size	SE	Pleiotropy
NYNRIN	rs8017377	6942	G/A (0.46)	0.03	A	0.030	0.045	No
ANXA9-CERS2	rs267733	6942	A/G (0.16)	0.033	A	-0.018	0.064	No
EHBP1	rs2710642	6942	A/G (0.35)	0.024	A	0.001	0.043	No
FN1	rs1250229	6942	C/T (0.27)	-0.024	T	0.020	0.046	No
BRCA2	rs4942486	6931	C/T (0.48)	0.024	T	0.000	0.040	No
SPTLC3	rs364585	6942	G/A (0.38)	-0.025	A	-0.006	0.042	No
SNX5	rs2328223	6942	A/C (0.21)	-0.03	A	-0.014	0.061	No
MTMR3	rs5763662	6942	C/T (0.04)	0.077	T	0.184	0.164	No
PCSK9	rs2479409	6942	A/G (0.32)	-0.064	A	-0.085	0.052	TC
SORT1	rs629301	6941	T/G (0.24)	0.167	T	0.054	0.051	TC
APOB	rs1367117	6942	G/A (0.32)	0.119	A	0.016	0.049	TC
ABCG5/8	rs4299376	6942	T/G (0.31)	-0.081	T	-0.004	0.047	TC
MYLIP	rs3757354	6942	C/T (0.24)	-0.038	T	0.000	0.049	TC
HFE	rs1800562	6941	G/A (0.07)	-0.062	A	0.055	0.087	TC
SLC22A1	rs1564348	6942	T/C (0.18)	-0.048	T	0.014	0.055	TC
PLEC1	rs11136341	6942	A/G (0.40)	-0.045	A	0.022	0.051	TC
ABO[*]	rs9411489	6942	C/T (0.21)	0.077	T	0.061	0.056	TC
ST3GAL4	rs11220462	6942	G/A (0.14)	0.059	A	0.015	0.063	TC
OSBPL7	rs7206971	6892	G/A (0.49)	0.029	A	0.008	0.042	TC
LDLR	rs6511720	6942	G/T (0.12)	-0.221	T	-0.123	0.079	TC
APOE	rs4420638	5642	A/G (0.19)	-0.225	A	-0.057	0.074	TC, HDL
TOP1	rs6029526	6942	T/A (0.47)	0.044	A	-0.024	0.042	TC
INSIG2	rs10490626	6942	G/A (0.08)	-0.051	A	-0.036	0.079	TC
LOC84931	rs2030746	6942	C/T (0.40)	0.021	T	-0.043	0.045	TC
CMTM6	rs7640978	6942	C/T (0.09)	-0.039	T	0.076	0.076	TC
ACAD11	rs17404153	6940	G/T (0.14)	-0.034	T	0.058	0.062	HDL
CSNK1G3	rs4530754	6942	A/G (0.46)	0.028	A	0.007	0.041	TC
MIR148A	rs4722551	6942	T/C (0.20)	-0.039	T	-0.013	0.063	TG, TC
SOX17	rs10102164	6942	G/A (0.21)	0.032	A	-0.027	0.050	TC
PPP1RB3	rs9987289	6942	G/A (0.10)	-0.071	A	-0.023	0.080	HDL, TC
CETP	rs3764261	6942	C/A (0.32)	-0.053	A	-0.033	0.045	HDL, TC, TG
LDLRAP1	rs12027135	6942	T/A (0.46)	-0.03	A	0.042	0.042	TC
MOSC1	rs2642442	6941	T/C (0.33)	0.036	T	0.034	0.047	TC
IRF2BP2	rs514230	6942	T/A (0.48)	-0.036	A	-0.035	0.042	TC
HMGCR	rs12916	6942	T/C (0.40)	-0.073	T	-0.067	0.043	TC
TIMD4	rs6882076	6942	C/T (0.36)	-0.046	T	0.059	0.044	TC, TG
HLA	rs3177928	6942	G/A (0.17)	0.045	A	0.034	0.059	TC
FRK	rs9488822	6942	A/T (0.36)	-0.031	A	-0.004	0.044	TC
DNAH11	rs12670798	6942	T/C (0.25)	-0.034	T	0.022	0.051	TC
NPC1L1	rs2072183	6942	G/C (0.29)	0.039	C	0.089	0.063	TC
CYP7A1	rs2081687	6942	C/T (0.36)	0.031	T	0.051	0.045	TC

Locus (closest gene)	SNP	n	Alleles Major/minor (MAF)	Weight	Coded Allele	Effect size	SE	Pleiotropy
GPAM	rs2255141	6942	G/A (0.30)	0.03	A	-0.024	0.046	TC
BRAP	rs11065987	6942	A/G (0.41)	0.027	A	-0.051	0.044	TC
HNF1A	rs1169288	6942	A/C (0.34)	-0.038	A	0.021	0.045	TC
HPR	rs2000999	6942	G/A (0.20)	0.065	A	0.044	0.056	TC
CILP2	rs10401969	6942	T/C (0.09)	0.118	T	0.121	0.087	TC, TG
MAFB	rs2902940	6942	A/G (0.30)	0.027	A	0.062	0.044	TC
ANGPTL3	rs2131925	6942	T/G (0.34)	0.049	T	0.068	0.044	TG, TC
TRIB1	rs2954029	6942	A/T (0.47)	0.056	A	0.050	0.042	TG,TC, HDL
FADS1-2-3	rs174546	6942	C/T (0.36)	-0.051	T	-0.099	0.044	TG, TC, HDL
APOA1	rs964184	6941	G/C (0.84)	-0.086	C	0.033	0.062	TG, TC, HDL
LRPAP1	rs6831256	6942	A/G (0.42)	0.025	A	-0.113	0.044	TG, TC
PIGV-NR0B2	rs12748152	6942	C/T (0.09)	0.05	T	-0.049	0.070	HDL, TG
UGT1A1	rs11563251	6942	C/T (0.12)	0.034	T	0.054	0.073	TC
VLDLR	rs3780181	6942	A/G (0.08)	0.044	A	0.099	0.072	TC
DLG4	rs314253	6942	T/C (0.37)	0.024	T	-0.059	0.044	TC
PPARA	rs4253772	6942	C/T (0.11)	-0.031	T	0.069	0.073	TC

The weights are the published effect sizes in SD units for LDL-C.[9] Effect size and SE correspond to the effect size of the coded allele and its standard error for AVC in the present study. [*]Proxy SNP rs495828 (ABO gene, r^2=1.0). *LPA* SNPs were excluded for established association with aortic valve disease.

eTable 2. Components of the Genetic Score for HDL-C in CHARGE

Locus (closest gene)	SNP	n	Alleles Major/minor (MAF)	Weight	Coded Allele	Effect size	SE	Pleiotropy
PABPC4	rs4660293	6942	A/G (0.24)	0.035	A	-0.008	0.049	No
ZNF648	rs1689800	6942	A/G (0.35)	0.034	A	0.036	0.044	No
COBLL1	rs12328675	6942	T/C (0.13)	-0.045	T	-0.015	0.062	No
SLC39A8	rs13107325	6911	C/T (0.08)	-0.071	T	-0.115	0.098	No
ARL15	rs6450176	6942	G/A (0.26)	-0.025	A	0.008	0.049	No
CITED2	rs605066	6942	T/C (0.42)	0.028	T	0.055	0.041	No
KLF14	rs4731702	6942	C/T (0.49)	0.029	T	0.036	0.043	No
TRPS1	rs2293889	6942	G/T (0.41)	-0.031	T	-0.041	0.042	No
AMPD3	rs2923084	6942	A/G (0.18)	0.026	A	0.111	0.055	No
LRP4	rs3136441	6942	T/C (0.18)	-0.054	T	-0.015	0.061	No
PDE3A	rs7134375	6942	C/A (0.43)	0.021	A	0.047	0.043	No
MVK	rs7134594	6942	T/C (0.48)	0.035	T	-0.032	0.042	No
SBNO1	rs4759375	6942	C/T (0.08)	0.056	T	-0.091	0.085	No
ZNF664	rs4765127	6939	G/T (0.35)	0.032	T	0.079	0.044	No
SCARB1	rs838880	6942	T/C (0.34)	-0.048	T	-0.009	0.045	No
LACTB	rs2652834	6942	G/A (0.21)	-0.028	A	-0.019	0.054	No
LCAT	rs16942887	6942	G/A (0.14)	0.083	A	-0.064	0.069	No
CMIP	rs2925979	6942	C/T (0.31)	-0.035	T	0.086	0.047	No
STARD3	rs11869286	6942	C/G (0.35)	0.032	C	-0.054	0.045	No
ABCA8	rs4148008	6942	C/G (0.33)	0.028	C	-0.015	0.045	No
PGS1	rs4129767	6942	A/G (0.48)	0.024	A	-0.095	0.042	No
MC4R	rs12967135	6942	G/A (0.25)	-0.026	A	0.072	0.049	No
ANGPTL4	rs7255436	6942	A/C (0.47)	0.032	A	-0.051	0.043	No
ANGPTL8	rs737337	6942	T/C (0.11)	0.056	T	-0.112	0.075	No
LILRA3	rs386000	6942	G/C (0.26)	-0.048	C	-0.086	0.059	No
UBE2L3	rs181362	6942	C/T (0.23)	-0.038	T	-0.134	0.054	No
HDGF-PMVK	rs12145743	6941	T/G (0.34)	-0.02	T	-0.036	0.047	No
ANGPTL1	rs4650994	6942	A/G (0.49)	-0.021	A	0.049	0.041	No
ATG7	rs2606736	6942	T/C (0.39)	-0.025	T	0.015	0.044	No
SETD2	rs2290547	6942	G/A (0.20)	-0.03	A	0.051	0.062	No
RBM5	rs2013208	6942	C/T (0.50)	0.025	T	-0.011	0.041	No
STAB1	rs13326165	6942	G/A (0.21)	0.029	A	-0.086	0.055	No
GSK3B	rs6805251	6942	C/T (0.39)	0.02	T	0.071	0.042	No
C4orf52	rs10019888	6942	A/G (0.18)	0.027	A	0.064	0.059	No
FAM13A	rs3822072	6942	G/A (0.46)	-0.025	A	0.044	0.043	No
ADH5	rs2602836	6933	G/A (0.44)	0.019	A	-0.029	0.041	No
DAGLB	rs702485	6942	A/G (0.45)	-0.024	A	-0.037	0.043	No
SNX13	rs4142995	6942	G/T (0.38)	-0.026	T	-0.026	0.042	No

Locus (closest gene)	SNP	n	Alleles Major/minor (MAF)	Weight	Coded Allele	Effect size	SE	Pleiotropy
IKZF1	rs4917014	6942	T/G (0.32)	-0.022	T	0.020	0.046	No
TMEM176A	rs17173637	6942	T/C (0.12)	0.036	T	-0.014	0.080	No
OR4C46	rs11246602	6942	T/C (0.15)	-0.034	T	0.032	0.064	No
KAT5	rs12801636	6942	G/A (0.23)	0.024	A	0.087	0.051	No
MOGAT2-DGAT2	rs499974	6937	C/A (0.19)	-0.026	A	-0.015	0.056	No
ZBTB42-AKT1	rs4983559	6942	A/G (0.40)	-0.02	A	-0.012	0.044	No
HAS1	rs17695224	6942	G/A (0.26)	-0.029	A	0.043	0.048	No
GALNT2	rs4846914	6942	A/G (0.41)	0.048	A	0.016	0.043	TG
IRS1	rs2972146	6942	T/G (0.37)	-0.032	T	-0.003	0.044	TG
PPP1R3B	rs9987289	6942	G/A (0.10)	-0.082	A	-0.023	0.080	TC, LDL
TTC39B	rs581080	6942	C/G (0.21)	0.042	C	0.049	0.055	TC
ABCA1	rs1883025	6942	C/T (0.25)	-0.07	T	-0.018	0.051	TC
LIPC	rs1532085	6942	G/A (0.40)	0.107	A	0.097	0.043	TC, TG
CETP	rs3764261	6942	C/A (0.32)	0.241	A	-0.033	0.045	LDL, TC, TG
LIPG	rs7241918	6942	T/G (0.19)	0.09	T	-0.025	0.053	TC
HNF4A	rs1800961	5641	C/T (0.05)	-0.127	T	-0.130	0.115	TC
PLTP	rs6065906	6942	T/C (0.19)	0.059	T	-0.067	0.052	TG
PIGV-NR0B2	rs12748152	6942	C/T (0.09)	-0.051	T	-0.049	0.070	LDL, TG
RSPO3	rs1936800	6942	T/C (0.49)	-0.02	T	-0.033	0.042	TG
MARCH8-ALOX5	rs970548	6942	A/C (0.26)	-0.026	A	-0.049	0.048	TC
FTO	rs1121980	6942	G/A (0.43)	-0.02	A	-0.062	0.043	TG
APOE	rs4420638	5642	A/G (0.19)	0.067	A	-0.057	0.074	LDL, TC
UBASH3B	rs7941030	6942	T/C (0.39)	-0.027	T	0.008	0.042	TC
MLXIPL	rs17145738	6942	C/T (0.13)	0.041	T	-0.066	0.065	TG
LPL	rs12678919	6942	A/G (0.13)	-0.155	A	0.124	0.072	TG
TRIB1	rs2954029	6942	A/T (0.47)	-0.04	A	0.050	0.042	TG, TC, LDL
FADS1-2-3	rs174546	6942	C/T (0.36)	-0.039	T	-0.099	0.044	TG, LDL, TC
APOA1	rs964184	6941	G/C (0.84)	0.106	C	0.033	0.062	TG, TC, LDL
LRP1	rs11613352	6942	C/T (0.26)	0.028	T	-0.029	0.047	TG
PEPD	rs731839	6942	A/G (0.35)	0.022	A	0.025	0.046	TG
VEGFA	rs998584	6941	C/A (0.49)	-0.026	A	-0.037	0.049	TG
ACAD11	rs17404153	6940	G/T (0.14)	0.028	T	0.058	0.062	LDL

The weights are the published effect sizes in SD units for HDL-C.[9] Effect size and SE correspond to the effect size of the coded allele and its standard error for AVC in the present study.

eTable 3. Components of the Genetic Score for TG in CHARGE

Locus (closest gene)	SNP	n	Alleles Major/minor (MAF)	Weight	Coded Allele	Effect size	SE	Pleiotropy
MSL2L1	rs645040	6940	T/G (0.23)	0.029	T	-0.025	0.047	No
KLHL8	rs442177	6942	T/G (0.42)	0.031	T	-0.075	0.043	No
MAP3K1	rs9686661	6942	C/T (0.20)	0.038	T	0.049	0.054	No
TYW1B	rs13238203	6942	C/T (0.04)	-0.059	T	0.132	0.137	No
PINX1	rs11776767	6942	G/C (0.37)	0.022	C	-0.069	0.043	No
JMJD1C	rs10761731	6942	A/T (0.44)	0.031	A	-0.003	0.043	No
CYP26A1	rs2068888	6942	G/A (0.45)	-0.024	A	0.024	0.044	No
CAPN3	rs2412710	6942	G/A (0.04)	0.099	A	-0.020	0.155	No
FRMD5	rs2929282	6942	A/T (0.07)	-0.072	A	0.021	0.104	No
CTF1	rs11649653	6942	C/G (0.40)	0.027	C	-0.006	0.044	No
PLA2G6	rs5756931	6942	T/C (0.40)	0.02	T	-0.004	0.044	No
MET	rs38855	6942	A/G (0.47)	0.019	A	0.047	0.042	No
AKR1C4	rs1832007	6942	A/G (0.18)	0.033	A	0.038	0.058	No
PDXDC1	rs3198697	6941	C/T (0.43)	-0.02	T	-0.008	0.043	No
MPP3	rs8077889	6938	A/C (0.22)	-0.025	A	-0.040	0.056	No
INSR	rs7248104	6942	G/A (0.42)	-0.022	A	-0.006	0.042	No
ANGPTL3	rs2131925	6942	T/G (0.34)	0.066	T	0.068	0.044	LDL, TC
GCKR	rs1260326	6942	C/T (0.39)	0.115	T	0.005	0.044	TC
MLXIPL	rs17145738	6942	C/T (0.13)	-0.115	T	-0.066	0.065	HDL
NAT2	rs1495741	6942	A/G (0.26)	-0.04	A	-0.063	0.052	TC
LPL	rs12678919	6942	A/G (0.13)	0.17	A	0.124	0.072	HDL
TRIB1	rs2954029	6942	A/T (0.47)	0.076	A	0.050	0.042	TC, LDL, HDL
FADS1-2-3	rs174546	6942	C/T (0.36)	0.045	T	-0.099	0.044	LDL, TC, HDL
APOA1	rs964184	6941	G/C (0.84)	-0.234	C	0.033	0.062	TC, HDL, LDL
LRP1	rs11613352	6942	C/T (0.26)	-0.028	T	-0.029	0.047	HDL
PEPD	rs731839	6942	A/G (0.35)	-0.022	A	0.025	0.046	HDL
LRPAP1	rs6831256	6942	A/G (0.42)	-0.026	A	-0.113	0.044	TC, LDL
VEGFA	rs998584	6941	C/A (0.49)	0.029	A	-0.037	0.049	HDL
GALNT2	rs4846914	6942	A/G (0.41)	-0.04	A	0.016	0.043	HDL
IRS1	rs2972146	6942	T/G (0.37)	0.028	T	-0.003	0.044	HDL
LIPC	rs1532085	6942	G/A (0.40)	0.031	A	0.097	0.043	HDL, TC
CETP	rs3764261	6942	C/A (0.32)	-0.04	A	-0.033	0.045	HDL, LDL, TC
PLTP	rs6065906	6942	T/C (0.19)	-0.053	T	-0.067	0.052	HDL
TIMD4	rs6882076	6942	C/T (0.36)	-0.029	T	0.059	0.044	TC, LDL
CILP2	rs10401969	6942	T/C (0.09)	0.121	T	0.121	0.087	TC, LDL
PIGV-NR0B2	rs12748152	6942	C/T (0.09)	0.037	T	-0.049	0.070	HDL, LDL
RSPO3	rs1936800	6942	T/C (0.49)	-0.02	T	-0.033	0.042	HDL
FTO	rs1121980	6942	G/A (0.43)	-0.021	A	-0.062	0.043	HDL
MIR148A	rs4722551	6942	T/C (0.20)	-0.029	T	-0.013	0.063	LDL, TC

The weights are the published effect sizes in SD units for TG.[9] The effect size and SE correspond to the effect size of the coded allele and its standard error for AVC in the present study.

eTable 4. Components of the Genetic Score for LDL-C in the MDCS

Locus (chromosome, closest gene)	SNP	Weight	Alleles (major/minor, MAF)	Risk allele	Pleiotropy
ANGPTL3	rs2131925*#	-1.59	T/G, 0.33	T	TG
IRF2BP2	rs514230	-1.13	A/T, 0.48	A	No
LDLRAP1	rs12027135	-1.1	T/A, 0.45	T	No
MOSC1	rs2642442*#	-1.09	T/C, 0.41	T	No
PCSK9	rs2479409	+2.01	A/G, 0.35	G	No
SORT1	rs629301	-5.65	A/C, 0.23	A	No
ABCG5/8	rs4299376	+2.75	T/G, 0.29	G	No
APOB	rs1367117	+4.05	G/A, 0.34	A	No
HMGCR	rs12916	+2.45	T/C, 0.41	C	No
TIMD4	rs6882076	-1.67	C/T, 0.36	C	TG
MYLIP	rs3757354	-1.43	G/A, 0.24	G	No
FRK	rs9488822#	-0.89	A/T, 0.31	A	No
HLA	rs3177928	+1.83	G/A, 0.13	A	No
HFE	rs1800562	-2.22	G/A, 0.05	G	No
DNAH11	rs12670798	+1.26	T/C, 0.25	C	No
NPC1L1	rs217386*	-1.17	C/G, 0.24	G	No
CYP7A1	rs2081687#	+0.95	C/T, 0.34	T	No
PLEC1	rs11136341*	+ 1.4	A/G, 0.38	G	No
PPP1R3B	rs9987289#	-2.22	G/A, 0.10	G	HDL
TRIB1	rs2954029#	-1.84	A/T, 0.47	A	HDL, TG
ABO	rs9411489	+2.24	G/A, 0.21	A	No
GPAM	rs2255141#	+1.08	G/A, 0.29	A	No
APOA1	rs964184	+2.85	C/G, 0.13	G	HDL, TG
FADS1	rs174546#	-1.71	C/T, 0.33	C	HDL, TG
ST3GAL4	rs11220462	+1.95	C/T, 0.14	T	No
BRAP	rs11065987	-0.97	A/G, 0.42	A	No
HNF1A	rs1169288	+1.42	T/G, 0.32	G	No
NYNRIN	rs8017377	+1.14	G/A, 0.46	A	No
CETP	rs3764261#	-1.45	G/T, 0.33	G	HDL, TG
HPR	rs2000999	+2.00	G/A, 0.21	A	No
OSBPL7	rs7206971*	+0.78	G/A, 0.48	A	No
APOE	rs4420638	+7.14	A/G, 0.20	G	HDL
CILP2	rs10401969	-3.11	T/C, 0.10	T	TG
LDLR	rs6511720	-6.99	G/T, 0.10	G	No
MAFB	rs2902940#	-0.98	A/G, 0.28	A	No
TOP1	rs6029526	+1.39	T/A, 0.48	A	No

A genetic score for low-density lipoprotein (LDL) cholesterol based on the 37 SNPs associated with LDL-C in the Global Lipids Genetics Consortium (GLGC) study.[11] Weights refer to effect estimates per minor allele in the GLGC study in mg/dl. Alleles and frequencies refer to the Malmö Diet and Cancer Study (MDCS). Pleiotropy refers to significant association with multiple loci in the GLGC. HDL, high-density lipoprotein cholesterol. TG, triglycerides. *LPA* SNPs were excluded for established association with aortic valve disease.*Failed QC. # Proxy SNP.

eTable 5. Components of the Genetic Score for HDL-C in the MDCS

Locus (chromosome, closest gene)	SNP	Weight	Alleles (major/minor, MAF)	Risk allele	Pleiotropy
GALNT2	rs4846914	-0.61	A/G, 0.40	G	TG
PABPC4	rs4660293	-0.48	A/G, 0.25	G	No
ZNF648	rs1689800	-0.47	T/C, 0.34	C	No
APOB	rs1042034	+0.90	A/G, 0.21	A	TG
COBLL1	rs12328675	+0.68	T/C, 0.12	T	No
IRS1	rs2972146	+0.46	A/C, 0.37	A	TG
SLC39A8	rs13107325	-0.84	C/T, 0.05	T	No
ARL15	rs6450176	-0.49	G/A, 0.25	A	No
C6orf106	rs2814944	-0.49	G/A, 0.16	A	No
CITED2	rs605066	-0.39	T/C, 0.44	C	No
KLF14	rs4731702*	+0.59	C/T, 0.49	C	No
MLXIPL	rs17145738	+0.57	C/T, 0.12	C	TG
LPL	rs12678919	+ 2.25	A/G, 0.09	A	TG
PPP1R3B	rs9987289	-1.21	G/A, 0.10	A	LDL
TRIB1	rs2954029#	+ 0.61	A/T, 0.47	A	LDL, TG
TRPS1	rs2293889	-0.44	G/T, 0.43	T	No
ABCA1	rs1883025	-0.94	G/A, 0.24	A	No
TTC39B	rs581080	-0.65	C/G, 0.19	G	No
AMPD3	rs2923084	-0.41	A/G, 0.17	G	No
APOA1	rs964184	-1.50	C/G, 0.13	G	LDL, TG
FADS1	rs174546#	-0.73	C/T, 0.33	T	LDL, TG
LRP4	rs3136441	+0.78	T/C, 0.14	T	No
UBASH3B	rs7941030#	+ 0.31	T/C, 0.39	T	No
LRP1	rs11613352#	+0.46	C/T, 0.27	C	No
MVK	rs7134594	-0.44	T/C, 0.46	C	No
PDE3A	rs7134375	+0.40	C/A, 0.43	C	No
SBNO1	rs4759377#	+0.86	C/T, 0.09	C	No
SCARB1	rs838880*	+0.61	T/C, 0.35	T	No
ZNF664	rs4765127	+0.44	G/T, 0.33	G	TG
LACTB	rs2652834	-0.39	C/T, 0.21	T	No
LIPC	rs1532085	+1.45	G/A, 0.38	G	TG
CETP	rs3764261	+3.39	G/T, 0.33	G	LDL, TG
CMIP	rs2925979	-0.45	G/A, 0.30	A	No
LCAT	rs16942887	+1.27	G/A, 0.14	G	No
ABCA8	rs4148008*	-0.42	G/C, 0.31	C	No
PGS1	rs4129767	-0.39	T/C, 0.48	C	No
STARD3	rs11869286	-0.48	C/G, 0.32	G	No
LIPG	rs7241918	-1.31	T/G, 0.17	G	No
MC4R	rs12967135	-0.42	G/A, 0.23	A	No
ANGPTL4	rs7255436	-0.45	A/C, 0.44	C	No
APOE	rs4420638	-1.06	A/G, 0.20	G	LDL
LILRA3	rs386000*	+0.83	C/G, 0.23	C	No
LOC55908	rs737337	-0.64	T/C, 0.11	C	No
HNF4A	rs1800961	-1.88	C/T, 0.04	T	No
PLTP	rs6065906*	-0.93	T/C, 0.18	C	TG
UBE2L3	rs181362	-0.46	G/A, 0.23	A	No

A genetic score for high-density lipoprotein (HDL) cholesterol based on the 47 SNPs associated with HDL-C in the Global Lipids Genetics Consortium study.[11] Weights refer to effect estimates per minor allele in the GLGC study in mg/dl. Alleles and frequencies refer to the Malmö Diet and Cancer Study

(MDCS). Pleiotropy refers to significant association with multiple loci in the GLGC. LDL, low-density lipoprotein cholesterol. TG, triglycerides. * Failed QC. # Proxy SNP.

eTable 6. Components of the Genetic Score for TG in the MDCS

Locus (chromosome, closest gene)	SNP	Weight	Alleles (major/minor, MAF)	Risk allele	Pleiotropy
ANGPTL3	rs2131925*	-4.94	T/G, 0.33	T	LDL
GALNT2	rs4846914#	+2.76	A/G, 0.40	G	HDL
APOB	rs1042034	-5.99	A/G, 0.21	A	HDL
COBLL1	rs10195252*	-2.01	T/C, 0.44	T	No
GCKR	rs1260326	+8.76	C/T, 0.37	T	No
IRS1	rs2972146#	-1.89	A/C, 0.37	A	HDL
MSL2L1	rs645040	-2.22	T/G, 0.22	T	No
KLHL8	rs442177	-2.25	A/C, 0.43	A	No
MAP3K1	rs9686661	+2.57	C/T, 0.16	T	No
TIMD4	rs6882076#	-2.63	C/T, 0.36	C	LDL
HLA	rs2247056	-2.99	G/A, 0.27	G	No
MLXIPL	rs17145738	-9.32	C/T, 0.12	C	HDL
TYW1B	rs13238203*	-7.91	C/T, 0.00	C	No
LPL	rs12678919	-13.64	A/G, 0.09	A	HDL
NAT2	rs1495741	+2.85	A/G, 0.22	G	No
PINX1	rs11776767	+2.01	G/C, 0.34	C	No
TRIB1	rs2954029	-5.64	A/T, 0.47	A	HDL, LDL
CYP26A1	rs2068888	-2.28	G/A, 0.44	G	No
JMJD1C	rs10761731*	-2.38	G/C, 0.46	G	No
APOA1	rs964184	+16.95	C/G, 0.13	G	HDL, LDL
FADS1	rs174546	+3.82	C/T, 0.33	T	HDL, LDL
LRP1	rs11613352	-2.70	C/T, 0.27	C	HDL
ZNF664	rs4765127#	-2.42	G/T, 0.33	G	HDL
CAPN3	rs2412710	+7.00	G/A, 0.02	A	No
FRMD5	rs2929282	+5.13	A/T, 0.04	T	No
LIPC	rs261342*	+2.99	G/A, 0.38	A	HDL
CETP	rs3764261#	-2.88	G/T, 0.33	G	HDL, LDL
CTF1	rs11649653	-2.13	C/G, 0.41	C	No
APOE	rs439401	-5.50	C/T, 0.36	C	No
CILP2	rs10401969	-7.83	T/C, 0.10	T	LDL
PLTP	rs6065906*#	+3.32	T/C, 0.18	C	HDL
PLA2G6	rs5756931	-1.54	T/C, 0.36	T	No

A genetic score for blood triglyceride (TG) concentration based on the 32 SNPs associated with TG in the Global Lipids Genetics Consortium study.[11] Weights refer to effect estimates per minor allele in the GLGC study. Alleles and frequencies refer to the Malmö Diet and Cancer Study (MDCS). Pleiotropy refers to significant association with multiple loci in the GLGC in mg/dl. HDL, high-density lipoprotein cholesterol. LDL, low-density lipoprotein cholesterol. * Failed QC. # Proxy SNP.

eTable 7. Genetic proxies in the MDCS

Trait	Chromosome	Gene	Original SNP	Proxy SNP	r^2	D'
HDL-C (n=5)	8	*TRIB1*	rs10808546	rs2954029	1.00	1.00
	11	*FADS1-2-3*	rs174601	rs174546	0.896	1.00
	11	*UBASH3B*	rs7115089	rs7941030	0.871	1.00
	12	*LRP1*	rs3741414	rs11613352	0.959	1.00
	12	*SBNO1*	rs4759375	rs4759377	1.00	1.00
TG (n=6)	1	*GALNT2*	rs1321257	rs4846914	0.965	1.00
	2	*IRS1*	rs2943645	rs2972146	1.00	1.00
	5	*TIMD4*	rs1553318	rs6882076	0.883	0.958
	12	*ZNF664*	rs12310367	rs4765127	0.965	1.00
	16	*CETP*	rs7205804	rs3764261	0.483	0.942
	20	*PLTP*	rs4810479	rs6065906	0.625	1.00
LDL-C (n=10)	1	*ANGPTL3*	rs3850634	rs2131925	0.965	1.00
	1	*MOSC1*	rs2807834	rs2642442	0.962	1.00
	8	*PPP1R3B*	rs2126259	rs9987289	0.803	1.00
	8	*TRIB1*	rs2954022	rs2954029	0.966	1.00
	11	*FADS1-2-3*	rs174583	rs174546	1.00	1.00
	16	*CETP*	rs247616	rs3764261	0.962	1.00
	20	*MAFB*	rs2902941	rs2902940	0.831	0.921
	6	*FRK*	rs11153594	rs9488822	0.676	0.954
	8	*CYP7A1*	rs1030431	rs2081687	0.845	0.919
	10	*GPAM*	rs1129555	rs2255141	0.959	1.00

Single nucleotide polymorphisms (SNPs) for which genetic proxies were genotyped and included in the genetic risk scores in the Malmö Diet and Cancer Study. Gene refers to the most proximal gene. Statistics (r^2 and D') describing the association of original SNPs with proxy SNPs are derived from the HapMap phase 2 CEU panel as made available using the Broad Institute SNP Annotation and Proxy Search (URL: http://www.broadinstitute.org/mpg/snap/ldsearchpw.php, accessed on 21th February 2013).

eTable 8. Correlations of genetic risk scores with plasma lipids in the MDCS

Genetic score	LDL-C	HDL-C	TG	Total cholesterol
LDL-C GRS	0.27 (0.24 to 0.29) p<0.001	-0.04 (-0.01 to -0.07) p=0.004	0.06 (0.04 to 0.09) p<0.001	0.24 (0.22 to 0.27) p<0.001
LDL-C GRS, (*non pleiotropic*)	0.21 (0.18 to 0.23) p<0.001	-0.01 (-0.04 to 0.01) p=0.31	0.02 (0 to 0.05) p=0.08	0.19 (0.16 to 0.21) p<0.001
HDL-C GRS	-0.04 (-0.01 to -0.07) p=0.005	0.24 (0.21 to 0.26) p<0.001	-0.10 (-0.07 to -0.12) p<0.001	0.03 (0 to 0.05) p=0.05
TG GRS	0.09 (0.06 to 0.12) p<0.001	-0.12 (-0.09 to -0.15) p<0.001	0.21 (0.18-0.23) p<0.001	0.10 (0.08 to 0.13) p<0.001

Pearson correlation coefficients for genetic risk scores with plasma lipids, and p-value. Confidence intervals were computed using Fisher's Z transformation. Cholesterol measures and genotypes were available in a random subset of the Malmö Diet and Cancer Study (MDCS, n=5269). A sensitivity analysis for the LDL-C score excluded SNPs also associated with HDL cholesterol or triglycerides in the Global Lipids Genetics Consortium.[11]

eTable 9. Association of LDL-C GRS with lipoprotein (a).

	AGES			FHS		
GRS	beta	stderr	Pval	beta	stderr	pval
LDL-C GRS	0.005	0.09	0.96	-0.15	0.13	0.22
LDL-C GRS specific	-0.05	0.10	0.61	-0.16	0.14	0.26

Results from linear regression analyses testing the association of each LDL-C GRS with log-transformed lipoprotein(a) in the two cohorts where measurements were available.

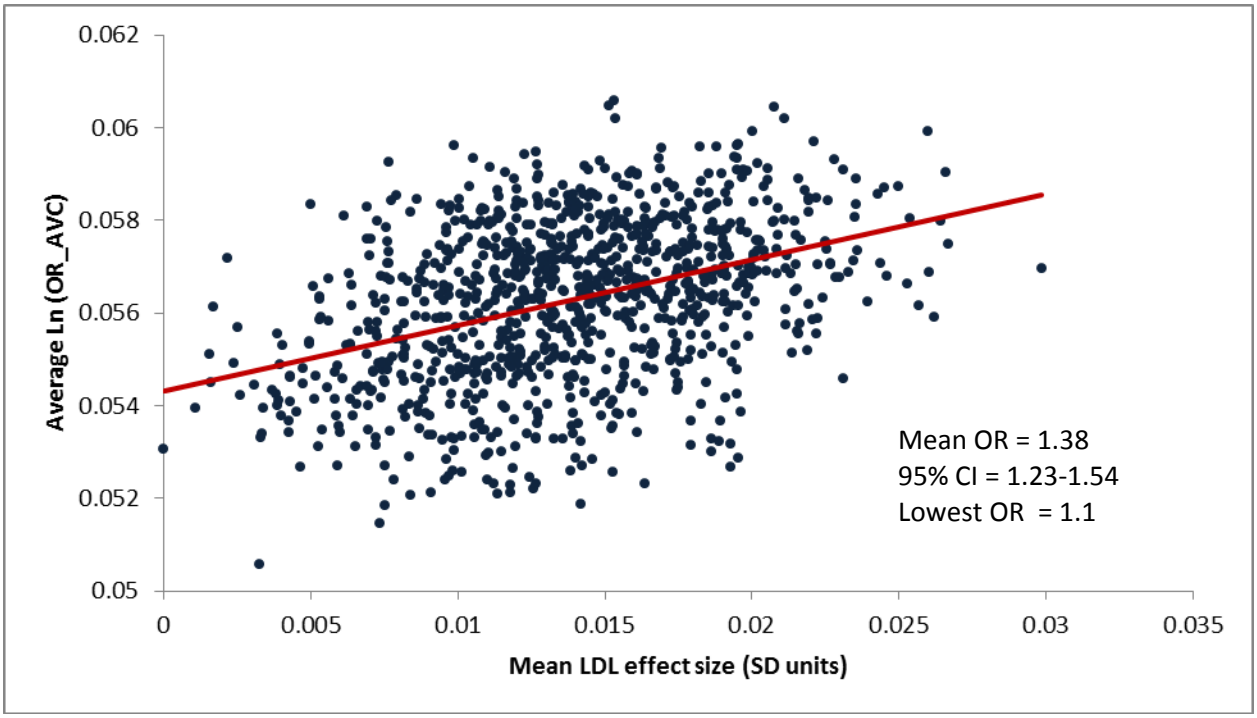
eTable 10. Mean and median Lp(a) by tertile of the LDL-C GRS

	AGES			FHS		
LDL GRS	N	Mean Lp(a)	Median Lp(a)	n	Mean Lp(a)	Median Lp(a)
1st tertile	960	9.4	5.1	738	14.9	8.8
2nd tertile	959	9.8	5.5	739	14.3	7.1
3rd tertile	959	9.3	4.9	739	15.6	7.6

eTable 11. Regression analyses of β_{AVC} on $\beta_{LDL\text{-}C}$, $\beta_{HDL\text{-}C}$ and $\beta_{TRIGLYCERIDES}$

Model	Outcome	Predictor	Covariate	Beta	SE	*P*
1	β_{AVC}	$\beta_{LDL\text{-}C}$	-	0.463	0.060	7.44E-13
2	β_{AVC}	$\beta_{LDL\text{-}C}$	$\beta_{HDL\text{-}C}$	0.464	0.060	6.99E-13
3	β_{AVC}	$\beta_{LDL\text{-}C}$	$\beta_{TRIGLYCERIDES}$	0.460	0.060	8.42E-13
4	β_{AVC}	$\beta_{LDL\text{-}C}$	$\beta_{HDL\text{-}C}$, $\beta_{TRIGLYCERIDES}$	0.467	0.059	3.23E-13
5	β_{AVC}	$\beta_{HDL\text{-}C}$	-	0.005	0.098	0.963
6	β_{AVC}	$\beta_{HDL\text{-}C}$	$\beta_{LDL\text{-}C}$	0.101	0.085	0.233
7	β_{AVC}	$\beta_{HDL\text{-}C}$	$\beta_{TRIGLYCERIDES}$	0.058	0.097	0.555
8	β_{AVC}	$\beta_{HDL\text{-}C}$	$\beta_{LDL\text{-}C}$, $\beta_{TRIGLYCERIDES}$	0.144	0.084	0.0878
9	β_{AVC}	$\beta_{TRIGLYCERIDES}$	-	0.141	0.113	0.2146
10	β_{AVC}	$\beta_{TRIGLYCERIDES}$	$\beta_{LDL\text{-}C}$	0.115	0.099	0.243
11	β_{AVC}	$\beta_{TRIGLYCERIDES}$	$\beta_{HDL\text{-}C}$	0.144	0.113	0.207
12	β_{AVC}	$\beta_{TRIGLYCERIDES}$	$\beta_{LDL\text{-}C}$, $\beta_{HDL\text{-}C}$	0.167	0.098	0.0882

eFigure 1 β_{LDL} vs β_{AVC} across 1000 simulated LDL-C GRS subsets by random exclusion of 30% of the LDL-C SNPs per subset



Mean OR, 95% CI and lowest OR for the association with AVC across all 1000 simulated LDL-C GRS are provided. As described, all 1000 simulated LDL-C GRS had an OR > 1.